ISSX NEWSLETTER

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Third International ISSX Meeting Amsterdam

(Contributed by Dr. Martin van der Graaff, Drug Disposition Department, Duphar B.V., Weesp, the Netherlands. Compiled partly from contributions by Session Chairmen).

The Third International ISSX Meeting, "Drug Metabolism: Molecules, Models and Man", was held June 24-28, 1991, at The Vrije Universiteit (Free University), in Amsterdam, The Netherlands.

The scientific program included two Short Courses, Plenary Lectures, Parallel Sessions, Poster Presentations and a Debate. Approximately 550 scientists from 28 countries around the world attended this meeting vouching for the truly international nature of ISSX. A total of 327 papers (course lectures excluded) were presented. The weather was, even to Dutch standards, extremely cold and wet, and this may have contributed to the enthusiastic participation and excellent attendance through the very last lectures on the last day of the meeting.

Short Courses

The scientific program began on Monday, 24 June, with two parallel Short Courses. Course I, organized by Dr. D.E. Case (Chairman, ISSX Committee on Regulatory Affairs) was entitled: **Drug metabolism and pharmacokinetics: Regulatory guidelines for the registration of pharmaceutical products - theory and practice.** Approximately 80 participants had pre-registered for this course, which seemed highly satisfactory. However, a similar number of meeting attendees registered for the course on Monday morning causing some rather crowded conditions within the lecture hall and a scramble for seats! Relocation to the main auditorium (Aula) in the afternoon solved the problem.

After a brief introduction to the regulatory scene by Dr. Case (ICI Pharmaceuticals, UK), the three main presentations focussed on the regulatory guideline material available in Europe, the USA, and in Japan.

The guidelines in Europe were reviewed by Dr. A. Bell (Glaxo, UK), who reviewed the procedures for regulatory submissions in the EEC, the preclinical and clinical guidelines, and the role of the Committee of Proprietary Medicinal Products (CPMP). He also described the requirement for an Expert Report and how this is constructed for European submissions.

The guideline position in the USA was reviewed by Dr. J. Dent (SmithKline Beecham, USA) who noted the emphasis in the USA on the correct formatting of regulatory submission material, rather than on detailed guidelines. He also commented on clinical bioavailability and bioequivalence studies and issues related to the use of drugs in the elderly, stereoisomerism, concentration-controlled clinical trials and toxicokinetics. He left us with the message that 'Guidelines do not supersede good science'.

The preclinical Japanese guidelines which were issued in January 1991 were discussed by Dr. H. Shindo (Sankyo, Japan), who also exemplified the types of questions posed by the Central Pharmaceutical Affairs Council (CPAC). He commented on the Japanese position related to chiral drugs, and drugs derived from cell culture technology, and illustrated the types of protocols adopted for typical preclinical radioisotope studies in Japan.

At the end of the Course, a Panel Forum was chaired by Dr. M. van der Waart (Organon, the Netherlands). Questions posed by the audience provoked considerable discussion, which was constrained only by the time available. Reflecting on the three rather different presentations made during the day by experts representing three major territories in pharmaceutical research and development, it would have been fascinating to challenge the speakers more closely on the prospects for harmonization in the future. But that must await another occasion!

Course II, organized by Drs. Nico Vermeulen and Peter van Bladeren was titled: **Computers in drug disposition studies**, and was attended by more than 75 participants. Recent developments in computer techniques and computer-based methods used in drug disposition research were reviewed, and concepts, theory and practical aspects were critically discussed by experts in this field.

Dr. F. Darvas presented the latest developments of computer-aided metabolism prediction using the MetabolExpert software. In addition to predicting potential biotransformations for compounds, this program also is capable of calculating hydrophobicity, HPLC-retention times and making interspecies predictions. HazardExpert can be used to predict toxic effects, bioaccumulation and

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bioavailability. In general, both programs are potentially useful; however, the general applicability seems as yet somewhat limited.

Dr. G. Donne discussed and evaluated molecular modeling and QSAR studies with enzyme and receptor systems. QSAR and distance geometry techniques have greatly contributed to the elucidation of the binding sites of the beta and histamine receptors. Additional molecular modeling and amino acid sequence alignment studies have allowed the further delineation of the tertiary shape of the binding site of a number of receptors and of new agonistic/antagonistic ligands. More recently, similar techniques have been used to model the bacterial cytosolic cytochrome P-450camphor and, interestingly, of the microsomal human cytochrome P-450 IID6, the poly-morphically regulated "debrisoquine P-450". A cytochrome P450 active site model with rather good predictive value for new substrates was described.

Dr. J. Hermens discussed the present status of QSAR studies in the field of environmental fate and effects of chemicals. Accumulation, bioconcentration and specific acute toxicities can be correlated using QSAR. The incorporation of even better models in the presently available databases and computer programs is a challenge for the future, particularly since these approaches are also being used by regulatory agencies (FDA, EPA).

Dr. H. Clewell demonstrated the potential of physiologically based pharmacokinetic modeling to examine bioactivation and disposition of xenobiotics. Investigation of specific aspects of disposition is used to refine the models: eg. methylene chloride, saturation of metabolism; trans-1,2-dichloroethylene, suicide enzyme inactivation; and allyl chloride, GSH depletion. The relative ease of use of such models was nicely demonstrated by a 'live' overhead presentation of the computer software in action.

Dr. J. van der Greef demonstrated that pattern recognition analysis using NMR or MS are interesting and useful tools for the optimal analysis of the enormous amount of information generated by these techniques. Examples were given for metabolic profiling, characterization of Salmonella strains (pyrolysis CI-MS), and characterization of migraine patients (EI-MS of urine). Computer programs incorporating multivariate and pattern recognition analyses are increasingly becoming available.

Overall, this course was appreciated by participants and speakers alike, as it achieved discussing these new computer methods in a broad perspective.

Opening Ceremony and Keynote Lecture

Following welcoming remarks by Dr. Gerard Mulder, Chairman of the Organizing Committee, the meeting was formally opened on Monday evening by Dr. E.H. Burger, Vice Rector of the Free University. Thereafter, Dr. F. Oesch, President of ISSX, delivered the keynote lecture, titled: **New vistas in xenobiotic metabolism in vivo and in vitro.** His talk was based, in part, upon the results of his own group. Dr. Oesch stressed that although molecular biology has greatly contributed to our understanding of xenobiotic metabolism, the challenge to understand drug disposition at the more integrated level of living cells, organs and animals is still enormous. If cell types differ in function, they also differ in their rates and routes of metabolizing xenobiotics. He noted that even within individual cell types, we still do not know all enzymes involved in drug metabolism, as illustrated by the recent discovery of a group of seven acidic glutathione transferases. He illustrated that new and very toxic metabolites (triol-epoxides) are still being discovered for well-studied compounds, the polycyclic aromatic hydrocarbons. The transfection of V79 cell lines with cytochrome P-450 DNA's results in cells that reproducibly express only one P-450 subspecies, and thus their relative importance in drug metabolism can be studied. These cells provide a reasonable reflection of hepatic metabolism, even though they have no bile secretion, if the results are compared with the sum of urinary and biliary excretion in vivo. Dr. Oesch concluded his lecture by urging all scientists in the audience to press on with our investigations, because ultimately both patients and the environment will benefit from our efforts.

Following the Welcoming Ceremony there was an enjoyable get-together in the auditorium hall and exhibition area.

Session 1: Drug Metabolism in Man

Dr. D. Breimer opened the first plenary session on Tuesday, June 25, by reviewing the various factors which contribute to variation in drug metabolism in man. By using a "cocktail" of suitable probe drugs, the existence of genetic polymorphism and the influence of genetic factors and conditions affecting enzyme activity can be assessed in one or two experiments. Compounds frequently used include antipyrine, sparteine, mephenytoin, nifedipine and indocyanine green; parent and metabolites are analyzed in blood and urine.

Dr. J. Miners discussed cytochrome P-450 in man, and focussed on P-450 IA2 and P-450 IIC. He explained that the 3-demethylation of caffeine is only a good probe for IA2 activity in human liver microsomes when low concentrations of substrate are used. The process can be inhibited by alpha napthoflavone. A low affinity component contributes to the 3-demethylation at higher concentrations of caffeine. An expressed human cytochrome P-450 IIC9 was shown to catalyze the hydroxylation of both tolbutamide and phenytoin. The kinetic and inhibition characteristics of the expressed enzyme were comparable to those of the human activities in vivo, indicating P-450 IIC9 is very likely the human isozyme responsible for the metabolism of phenytoin and tolbutamide.

Dr. R. Verbeeck discussed conjugation in man, in particular of diflunisal, which forms an acyl glucuronide (DAG), a phenolic glucuronide (DPG) and a sulphate (DS) among other metabolites. Alterations of the relative contributions of these metabolites to overall elimination of diflunisal were explained for increasing doses

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(decreased clearance as glucuronides), gender (young women show reduced formation of DPG), use of contraceptives or smoking in women (increased glucuronidation), hepatic cirrhosis (reduced glucuronidation), and renal failure (systemic deconjugation of DAG, increased biliary excretion of glucuronides). Sulphation was less perturbed by probenecid administration than glucuronidation.

Dr. D. Harrison described immunohistochemical studies to determine the distribution in human tissues of alpha, mu and pi cytosolic glutathione S-transferase (GST) and microsomal GST. In general, secretory or duct type epithelium express GST-pi. In contrast, in parenchymal cells GST-alpha predominates. The effect of various diseases on the distribution within organs and cell types was illustrated. Cause and effect are not yet clear; eg. although GST is implicated in tumor cell drug resistance, some tissues express little GST, whereas other tissues show increased GST activity.

Dr. U. Meyer lectured on arylamine N-acetyltransferases (NATs) and explained that NAT1 is an ubiquitous monomorphic protein with a high affinity for substrates such as p-aminobenzoic acid. In contrast, NAT2 predominantly is expressed in the liver, demonstrates polymorphism and has a high affinity for substrates such as sulphametazine. Remarkably, rapid and slow acetylators have similar NAT2 mRNA levels, but the protein is drastically reduced in slow metabolizers. Four different mutation types in the slow metabolizer alleles have been identified that cause decreased NAT2 by different mechanisms. It is possible to correctly identify by a DNA test greater than 95% of mutant alleles and predict the phenotype.

Session 2: Species and Sex Differences from Animal to Man

The first parallel session was opened Tuesday afternoon by Dr. J. Caldwell who discussed the (ir)relevance of interspecies differences in safety assessment of drugs and food additives. Two specific examples were presented. The site and stereospecificity of propyl chain hydroxylation of 6-propyl-chromone-2-carboxylic acid by different species was discussed as was the effect of specific cytochrome P450 inducers. For the ester cinnamyl anthranilate, the toxicity (ie. induction of lauric acid hydroxylase and peroxisome proliferation) was shown to be dependent on the presence of unchanged compound. Since it was demonstrated that only the rat fails to completely hydrolyze this food additive, the safety extrapolation from rat to man which resulted in a ban on this compound was retrospectively shown to be incorrect.

Dr. Y. Yamazoe gave a comprehensive overview of sex differences in metabolism, mainly in the rat, and explained which subspecies of cytochrome P-450 are suppressed or induced by growth hormone, a major factor in determination of sex differences in metabolism. The effect of growth hormone administration on the cytochrome P-450 composition in dwarf rats was demonstrated. It was pointed out that non-P-450 enzymes also show sex-differences in rat liver, such as the cytosolic sulfotransferases which catalyze sulfation of steroids. Alcoholic steroids are predominantly sulfated in females, while phenolics are sulfated by males. Currently no good explanations are available to explain the fact that clear sex-differences in metabolism are not found in man.

Dr. F. Belpaire indicated that differences in protein binding between species can sometimes be related to differences in methodology. Acidic drugs, which mainly bind to albumin, are in general more extensively bound to serum in humans than in other mammals. The effects of different concentrations of albumin and different binding affinities were discussed. For drugs mainly bound to AAG the interspecies differences in binding are more pronounced, but literature data are far from conclusive. Some examples of enantioselective binding were given, but these generally do not have serious therapeutic consequences.

Dr. N. Grubb described disposition studies with indobufen, a platelet aggregation inhibitor, which is administered as the racemate although only the S-enantiomer is active. The disposition of both the racemate and the S-enantiomer was studied in rats, mice and human volunteers. The disposition of the isomers was different in the species examined.

Dr. W. Meuldermans discussed the disposition of the novel antipsychotic drug, risperidone, in rats, dogs and man. The extent of urinary and fecal excretion varied considerably between species, although the metabolite patterns were comparable. The formation in man of the major metabolite in plasma, 9-hydroxy-risperidone, was subject to a debrisoquine-type polymorphism.

Session 3: Food and Drug Metabolism

The effect of several food constituents on the metabolism of xenobiotics in liver and by intestinal flora was discussed in this session.

Dr. I. Rowland presented recent results which demonstrated that non- or poorly fermentable dietary fibers, such as carrageenan, strongly depress intestinal microbial reductive reactions, whereas fermentable fibers, such as pectin, have the opposite effect. Changes observed after consumption of high-fat diets appear to originate mainly from increased levels of bile acids in the intestine.

Dr. C. Yang discussed the cytochrome P-450 induction caused by dietary substances such as diallyl sulfide (DAS) in garlic and indole-3-carbinol in cruciferous vegetables. DAS induces cytochrome P-450 IIB1 but inactivates P-450 IIE1 by suicide inhibition. Phenethyl isothiocyanate from cruciferous vegetables also is a suicide substrate for P-450 IIE1. These actions may modulate carcinogenic or cytotoxic effects of various compounds (eg. nitrosamines or aflatoxin) in the liver.

Dr. H. Verhagen proposed that BHA-induced forestomach tumors are caused by formation of quinone and sulfur containing metabolites, which may generate reactive oxygen species.

Dr. S.M. Sanins discussed the fate of

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Amsterdam
June 24 - 28, 1991

1. Ron Estabrook and Franz Oesch discuss the day's events.
2. Peter van Bladeren and Martin van der Graaff reminisce.
3. Monday morning registration queue blues.
4. Mitch Cayen, Tom Baillie and Bob Smith between sessions.
5. Regulatory short course panel members: Hideyo Shindo, John Dent, M. van der Waart and Angus Bell.
6. David Case and John Dent continue regulatory discussions after the course.
7. Gerard Mulder welcomes the attendees to the Third International ISSX meeting.

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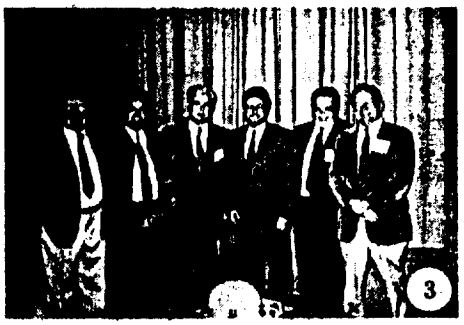
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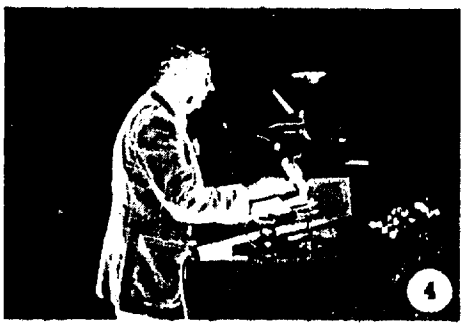
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1. Heineken socialization-stimulating factor.
2. Peter van Bladeren, Richard Armstrong, Nico Vermeulen and Harvey Clewell discuss the Computers short course.
3. The Cytochrome P450 IAI debate participants: Dennis Parke, Patrick Maurel, David Case, Andrew Parkinson, John Caldwell and Ron Estabrook.
4. Ron Estabrook defines the debate rules of order.
5. Andrew Parkinson and John Caldwell plan debate strategy.
6. Dennis Parke continues discussions with one of his former postdoctoral fellows, Chris Rhodes.
7. Franz Oesch delivering the keynote lecture.

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orally administered Saccharomyces boulardii (Sb), used for the treatment of antibiotic-associated diarrhea. Recovery of the patients was increased by simultaneous treatment with ampicillin, indicating that the intestinal flora is involved in Sb elimination.

Dr. T. Borlakoglu reported a mutual synergistic effect of 3,3',4,4'-tetrachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl on the induction of cytochrome P-450 IVA1, after lactational transfer to neonates. In the same animals, peroxisomal beta-oxidation and fatty acid CoA oxidation were reduced rather than induced as seen after treatment with other P-450 IVA1 inducers, such as hypolipidemic drugs.

Session 4: Analytical Techniques and Approaches

Dr. J. van der Greef highlighted the use of LC/MS in studies on xenobiotic metabolism. He emphasized that different types of interfaces (thermospray, electrospray) and ionization techniques are available and all have their specific advantages depending on the analyte of interst and the eluent used in the LC. Dr. van der Greef presented numerous practical examples to underscore his message.

Dr. Brinkman illustrated that pre-column events (in particular enrichment of analytes) as well as post-column events (preferably photochemical or heterogeneously-catalyzed reactions which do not require an additional pump) can improve speed, sensitivity and selectivity of LC analysis.

Dr. L. Delbressine presented in his paper the powerful combination of NMR and mass spectroscopy to identify uncommon and well-known conjugates such as N+-glucuronides, N-sulfates, N-O-glucuronides and N-carbamate-glucuronides, and the correct assignment of regio- and stereoisomers.

From protein analytical techniques (enzymatic digestion and HPLC of the peptides) combined with mass spectroscopy, Dr. J. Cashman was able to determine that the flavin-containing monooxygenase (FMO) from rabbit lung was present as a complex with calreticulin, thus explaining the peculiar properties of the lung enzyme compared to the liver FMO.

Finally, Dr. S. Nagatsuka demonstrated the capabilities of the Bioimage Analyzer, a two-dimensional radio-activity monitor which is approximately 100-times more sensitive than X-ray films. 14C-Diazepam and metabolites could be detected with high sensitivity in biological fluids.

The day finished with a boat trip from a site near the University to the Scheepvaart Naval History Museum via the most picturesque Amsterdam canals. The reception at the museum, hosted by Mr. Van den Berg (a member of the Council of Amsterdam), was enjoyed by all. Between drinks, the historical collection was admired.

Session 5: Molecular Mechanisms and Toxicology

This second plenary session was held Wednesday morning, July 26.

Dr. T. Baillie described a number of mechanisms by which biotransformation produces metabolites which are toxic to cytochrome P-450 or other cell constituents. N-methyl-formamide is metabolized to methyl isocyanate. Reversible glutathione conjugation results in a transport form, which upon deconjugation may react with thiol groups of macromolecules, resulting in toxic and/or antitumor effects. Detailed studies on the chiral inversion of stiripentol showed that the mechanism is not analogous to that for ibuprofen, but rather is the result of the loss of the sulphate conjugate, leading to a positively charged carbon atom. An understanding of metabolism can lead to the design of analogues which do not require metabolic activation or which lack toxicophores.

Dr. F.P. Guengerich described the pathway leading to adduct formation between dihaloalkane metabolites and DNA bases. The structure of major and minor adducts was elucidated, and an unstable GS-CH2-CH2-X conjugate (a sulfur half-mustard) was postulated as the reactive intermediate. A synthetic route to prepare these compounds was presented. The toxicity of the dihaloalkanes is increased upon induction of glutathione S-transferase or inhibition of cytochrome P-450. DNA sequencing techniques indicated that most of the induced mutations were GC/AT transitions. However, the most frequently found DNA adducts were shown to be less relevant toxicologically than the minor adducts, but the latter can be measured in the urine as guanine mercapturic acids to provide a biomonitor of exposure.

Physiological modelling was demonstrated by Dr. H. Clewell to be a powerful tool for assessing the time course of toxic compounds in the target tissue. This approach, which studies the whole organism, enables a relevant risk assessment, since it also takes into account capacity limited processes, perfusion limitations, and induction/inhibition. As an example, the disposition of volatile haloalkenes using a closed chamber system was illustrated as was the use of experimental data to refine the model.

The effects of free radicals were presented by Dr. A. Bast. Radicals can be generated, for example, during reperfusion after ischemia. One of the ultimate consequences on the cell of the process called 'oxidative stress' is calcium influx with concomitant loss of intracellular calcium homeostasis. The effects of free radicals on the responsiveness of pharmacological receptors were also discussed. Dr. Bast identified three lines of defense against radicals: 1) scavenging of initiators; 2) chain-breaking antioxidants; and 3) metabolism of lipid peroxidation products. New antioxidant drugs are presently under development, such as ebselen, 2-octadecyl ascorbic acid, vitamin E analogues and lazaroide.

Dr. D. Henschler stressed the need for mechanism-based hazard assessment with newly developed chemicals. Elucidation of the mechanism of action on the cellular and molecular level is necessary for interspecies comparisons and extrapolation to man. These comparisons should be based on the respective reaction rates of the

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individual bioactivation steps.

Session 6: Enzyme Mechanisms, Active Sites, and SAR

Two physical methods, NMR and X-ray crystallography, were discussed extensively during this session, as well as studies with series of related substrates to probe the boundaries of the active site and catalytic mechanism.

Dr. P. Ortiz de Montellano described his work with cytochrome P-450. Because crystallization of mammalian cytochrome P-450's has not been possible so far, he has compared the regio- and stereoselectivity of the oxidation of styrene and various substituted analogues by both cytochrome P-450camphor and cytochrome C-oxidase. Molecular dynamics calculations (in cooperation with Dr. G. Loew) correctly predict the stereochemistry of the metabolism of these substrates by P-450camphor.

Dr. R. Armstrong discussed the glutathione S-transferases, particularly studies on the crystal structure of rat isoenzyme 3-3. Presently two thirds of the enzyme backbone has been mapped, and the full structure is expected shortly. Interestingly, the two active sites of this dimeric enzyme are located along the side where the two subunits are connected. Neither histidine nor cysteine residues appear to play an essential role in the catalytic mechanism of this enzyme as determined with a series of mutant enzymes in which each of the four histidine or the four cysteine residues had been selectively deleted.

Dr. W. Alworth presented his work on the mapping of the active site of cytochrome P-450 IA1 using a series of ethynyl-substituted pyrenes. These compounds act as irreversible inhibitors, and from the rate of inhibition it has been possible to draw conclusions about the binding affinity of the substrates.

Dr. T. Satoh reviewed his extensive work on the characterization of a large series of carboxylesterases from several animal species and man, and Dr. D. Ross described his elegant work with DT-diaphorase using mitomycin C as a substrate.

Session 7: Idiosyncratic Reactions

The mechanisms responsible for idiosyncratic drug reactions were discussed in this parallel session. These adverse effects are problematic for the pharmaceutical industry, since they sometimes occur only after continued clinical use of a drug.

Dr. L. Pohl stressed that the majority of hepatotoxic drugs appear to be idiosyncratic hepatotoxins. He discussed the mechanism of halothane-induced hepatitis: it has been demonstrated that the trifluoroacetyl metabolite of halothane is bound covalently to several liver proteins which is believed to result in an antigenic response.

Dr. B. Park illustrated newly developed in vitro techniques incorporating human cells and tissues to detect and characterize stable, chemically reactive and cytotoxic metabolites. Specific examples with dapsone, carbamazepine and amodiaquine were discussed. The mechanisms leading to drug-antibody formation are now generally understood. However, it is unclear why some patients who produce antibodies do not demonstrate any toxicity.

Dr. D. Mansuy uses human hepatic cytochrome P-450's of the IIC subfamily expressed in yeast to study the 5-hydroxylation and metabolic activation of tienilic acid. A new class of highly unstable, reactive intermediates (thiophene sulfoxides) has been discovered using this system.

Dr. R. Dickinson demonstrated that diflunisal glucuronides interact with albumin, but transacylation is not the mechanism of formation of covalent adducts in vitro.

Finally, Dr. V. Fischer discussed the possible role of radical formation in clozapine-induced agranulocytosis. The glutathionyl radical was identified by using a spin trapping technique. Both horseradish peroxidase and myeloperoxidase/hydrogen peroxide (models for oxidation processes in neutrophils) generate radical intermediates of clozapine. Adduct formation of radical intermediates may be the final step resulting in agranulocytosis.

Session 8: In Vitro Biotransformation Models

In this well-attended session, Dr. V. Rogiers discussed the use of hepatocyte cultures as model systems for biotransformation studies. The use of in vitro systems including liver perfusion, tissue slices, subcellular fractions, shaking cultures and primary cultures has increased during the past decades and has the benefits of reduced use of animals and rapid generation of results. Systems containing intact cells are preferable. However, optimal cell culture conditions for the maintenance of metabolic capacity have not yet been established. Culture conditions can be improved by supplementing with soluble factors (hormones, growth factors, etc.), by addition of an extracellular matrix and by co-culture with other cell types. Dr. Rogiers described a system in which rat and human hepatocytes were co-cultured with bile duct epithelial cells. A number of liver specific functions were stabilized in rat hepatocytes for a longer period, however, co-culture did not cause any improvement for human hepatocytes.

Dr. J. Doehmer described the use of genetically engineered cells in drug metabolism. Full length cDNA's for different rat and human cytochrome P-450's have been cloned and transfected to V79 cells (which usually do not express P-450) resulting in highly metabolically active cells. Potential applications of these cells in pharmacology and toxicology studies were discussed. This approach may help answer specific questions related to xenobiotic metabolism and toxicity, and occupies an intermediate position between the most complex (man) and simplest (isolated enzymes) systems.

The use of transgenic animals was reviewed by Dr. W. Beltz. He defined them as "organisms with artificially introduced genetic material" and described the ethical considerations, the available technology and its pitfalls. Desirable properties of the introduced genetic material include: non-lethality, stable expression, a good characterization,

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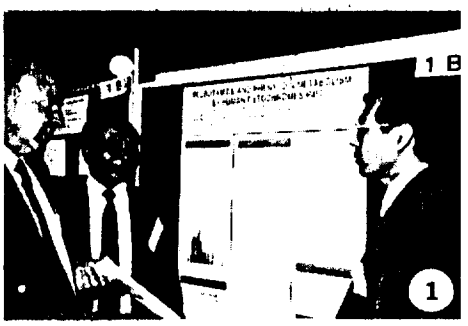

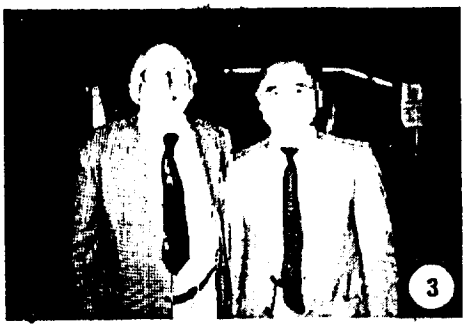

1. Thoughtful discussions at the poster sessions.
2. Dhiren Thakker and Mitch Cayen discuss a point.
3. Dennis Parke and Ryuichi Kato after the P450 IAI debate.
4. Enroute to the welcoming reception at the Scheepvaart Museum.
5. Hideyo Noguchi, Tetsuya Suga and Hideyo Shindo enjoy the boat ride.
6. Gerard Mulder, Nico Vermeulen, Marije van Urk (Conference Service), Barbara Oesch-Bartlomowicz and Franz Oesch with Mr. Van den Berg (2nd from left), Amsterdam Council Member.
7. At the Scheepvaart Museum.

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1. Franz Oesch presents a plaque to Bruce Midgalof, one of the four Founders of ISSX, while Nancy Holahan looks on.
2. Franz Oesch presents a certificate to Past-President Ron Estabrook.
3. President-Elect Mitch Cayen (right) presents a plaque to outgoing President Franz Oesch.
4. David Case and Ron Estabrook enjoy the conference banquet festivities.
5. Dance fever at the banquet.
6. ISSX Council meeting.
7. Amsterdam.

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and the presence in the germ line, so that the progeny can be used. This laborious technique can take as long as a year to produce offspring that can be used in experiments. He described the inclusion of a number of oncogenes in mice and their use in the study of anticancer drugs.

Dr. A. Kempf discussed the expression of cytochrome P-450 IIG1 in E. coli. This P-450 is specifically expressed in the olfactory mucosa of the rat and the exact role of this isozyme still has to be established. The characteristics of the expressed protein were compared with those of the rat enzyme. This system can be used as a source of protein for characterization and for the production of specific antibodies.

Dr. P. Nasseri-Sina presented data on the biotransformation activity of human and rat keratinocytes in culture. Such cultures can now be used to study the metabolism, cytotoxicity, irritation, and percutaneous absorption of compounds. Several metabolic activities were determined including ECOD, EROD, esterases and ADH.

DEBATE: Does induction of Cytochrome P-450 IAI indicate potential carcinogenicity?

Dr. R. Estabrook was the organizer and moderator Wednesday evening of this debate which he described as "an experiment". After Dr. Estabrook explained the rules of order for the debate, Dr. D. Case presented the dilemma confronting industry: "Is it correct or not to throw away an investigational compound once it has been proven to induce P-450 IA1?"

The "pro" position was defended by Drs. D. Parke and P. Maurel, while Drs. A. Parkinson and J. Caldwell argued "contra". Protagonists and antagonists took turns in propounding their arguments. The debate was lively and interesting. Some of the points made by the "pro" side included: most carcinogens are planar compounds and planar compounds induce P-450 IA1; there is a high correlation between lung cancer incidence and IA1 concentration in patients; a modest induction in relatively insensitive animals sometimes turns out to be more dangerous than a strong induction in sensitive animals. This can be explained assuming a threshold for carcinogenesis: nature has down- regulated P-450 IA1 in man after birth, and therefore we should at all costs avoid re-expression of this enzyme.

Some of the "contra" arguments included: induction of P-450 IA1 has been shown to be deleterious in vitro, but not in vivo; there are grounds to defend that P-450 IAI induction may even be beneficial in vivo; P-450 IIB and IA induction are no different as both induction curves are S-shaped and have a threshold; usually there is only an association between IA1 induction and carcinogenesis; to single out only P-450 IA1 inducers is not logical since inducers of other P-450's are also associated with toxicological effects.

Following the presentations and summations, the audience was asked to vote. The response was overwhelmingly in favor of the "contra" position. The "experiment" was generally considered a success, thanks to the enthusiastic involvement of all debaters and hopefully similar events will be held at future meetings on equally important and interesting topics.

(Editor's note: A more detailed review of the debate will be included in the next issue of the Newsletter)

Session 9: **Transport of Xenobiotics and Metabolites**

The Thursday plenary session was opened by Dr. D. Meijer who gave a talk on hepatic transport mechanisms. Uptake in the liver can be mediated via passive diffusion, adsorptive endocytosis and carrier mediated transport. Examples were given and the effect of specific zonal destruction on uptake was described. Carriers exist within the cells for both anions and cations. The concentration of albumin in the perfusion medium has an effect on excretion: in the absence of albumin, excreted material will be reabsorbed downstream. Two 50 kD sodium dependent multifunctional anion carriers were described, as was a 100 kD carrier though to be responsible for transport into the bile.

Dr. M. deBroe discussed the anatomy and function of the kidney in relation to the handling of several classes of compounds by this organ. He reviewed two compounds for which knowledge of their renal handling enabled optimization of drug therapy. Imipenem is an antibiotic that is degraded intraluminally by brush border enzymes. Co-administration of cilastatin results in a decrease in tubular excretion of imipenem and inhibition of the brush border enzymes. Aminoglycosides, which are positively charged at physiological pH, bind to negatively charged phospholipids in lysosomes. Continuous administration of aminoglycosides results in greater accumulation and hence toxicity to the lysosomes. Therefore pulsed administration is preferable to infusion.

Dr. F. Teeuwes gave an overview of the factors which affect absorption of drugs through skin. The epidermis is the most important barrier. The increase in absorption when using a chemical enhancer is a function of solubility of the drug in the enhancer and solubility of the enhancer in the skin. The application of an electrical current to the skin also has an enhancing effect. In some cases, obtaining target concentrations in the blood using electrotransport can be achieved as rapidly as with an i.v. infusion.

Pulmonary uptake systems were discussed by Dr. G. Cohen. He focused on the uptake system for paraquat, which is concentrated by Type 2 cells and epithelial Type 1 cells. Paraquat is transported by a carrier for endogenous polyamines, such as putrescine and spermidine. Positive charges must be 4 to 7 angstroms apart, and the nitrogens of paraquat meet this requirement. Cystamine inhibits this transport process, but is itself quickly converted to taurine, for which a separate uptake system exists. Since tumor tissues have comparable polyamine carriers, putrescine analogues coupled to cytostatics could be used for targeted drug therapy.

Dr. J. Tillement stressed that albumin and alpha-1 acid glycoprotein (AAG) are not the only blood components

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that bind drugs. Some lipid-soluble drugs equilibrate rapidly with the lipids in the red cell membrane. For highly protein-bound drugs, Dr. Tillement made a distinction between restrictive binding which results in a low volume of distribution (<0.6 L/kg) and a low clearance (eg. for butazolidine), and permissive binding which results in a higher volume of distribution and higher clearance (eg. for chlorpromazine). A therapeutic consequence was given for patients treated with azapropazone. Although the overall plasma binding can be the same as in healthy volunteers, since patients have lower albumin levels more drug is permissively bound to AAG, and more drug is available for tissue penetration.

Symposium Dinner

Thursday afternoon was free and many participants took the opportunity to see the sights or do some shopping. The symposium dinner was held Thursday evening in a very relaxed atmoshpere in the Breughelhuys, where the 16th century was evoked by the songs of troubadours, the presence of a clog-maker and staff dressed in historic costumes.

In a short ceremony, Dr. Franz Oesch honored the dedication of several ISSX members. Noting that 1991 marks the Society's tenth anniversary, Dr. Oesch praised our four Founders, John Baer, Fred Di Carlo, Bruce Migdalof and Ina Snow (deceased) for their foresight and vision, and they were honored with commemorative plaques. Dr. B. Migdalof was present and received his plaque in person; Drs. Baer and Di Carlo were not present, but sent messages which were read by Dr. Oesch. Dr. Oesch also recognized the efforts of the past Presidents of ISSX, Drs. R. Smith, R. Kato, and R. Estabrook. Each will receive an award; Dr. Estabrook was present and received his award that evening. Outgoing ISSX Officers were also honored: Dr. F. Pritchard (Treasurer), who was present, and Dr. D. Yesair (Secretary), who was not present. Finally, outgoing President Dr. Oesch was presented an award by President-Elect, Dr. M. Cayen.

Session 10: Xenobiotics and the Environment

Dr. H. Poiger opened Friday's plenary session by discussing the fate of chlorinated dioxins and dibenzofurans in the environment and living tissues. The laterally (2,3,7,8) chloro-substituted dioxins have the highest toxicity and accumulate in the human food chain. Soil half-life is in the order of ten years, since microbial metabolism is virtually absent. Rat and dog metabolize dioxins extremely slowly to much less toxic compounds, via reductive dechlorination, hydroxyl-ation, and rarely, ring scission. In rats, metabolism is auto-inducible. The half-life in animals ranges from 20 days in rats and mice to approximately 10 years in man. In rats accumulation is mainly in liver (isomer-specific) while in man accumulation is mainly in fat (irrespective of isomer).

Dr. S. Safe discussed the molecular events in the sequence leading to induction of rodent hepatic P-450 IA1 and 1A2 by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Binding of TCDD to the cytosolic Ah receptor is the first step. A systematic investigation eliminated several mechanisms as explanations for the known structure-activity relationship with dioxin analogues. It was concluded that there is a correlation between Kd values (k[reverse]/k[forward]) for initial receptor ligand interaction versus induction potencies.

A. Zehnder reviewed the fate of a number of organic compounds in microbial cultures. Frequently, these are mineralized, ie. metabolized to CO2, H2O, and HCl. The pathways depend on species and strain, and reaction rate has a temperature optimum around 30C, which limits the use of bacteria as environmental cleaners. Some pathways specific for microbes include anaerobic reductive dehalogenation, and ring scission (eg. of 2,3,-dihydroxy-chlorobenzene) which may lead either to a dicarboxylic acid or an acyl chloride (suicide pathway).

Dr. D. Hutson compared and contrasted metabolic pathways in plants and animals. Some similarities exist, but there are also differences (eg. plants make glucosides rather than glucuronides). Within the plant kingdom there are species differences in metabolism, and this provides the basis for selective crop protection against weeds. Neither plants nor insects contribute significantly to to the degradation of crop protection agents. With the modern generation of these agents, however, this is not a problem, since the residue levels are extremely low and trace analysis is the real problem (levels of 0.07 ppm are not uncommon).

Dr. T. Roberts took the audience into the world of real-life field studies with xenobiotics. He described the use of the lysimeter that enables transfer of blocks of soil 1 meter deep and 0.5 meter across to the laboratory. The advantage is that climatic processes can be controlled in the laboratory, although care should be taken to account for the loss of sun irradiation, which may cause decomposition of some compounds. He also stressed that permeation of compounds through the soil may be a dose-dependent process. One difficulty in this discipline is the country-to-country variability in the favored approach, which ranges from field studies in the USA to computer simulations in the Netherlands.

Session 11: Disposition of Protein and Peptides

Within this session, analytical, regulatory, and dispositional aspects of peptides and proteins were discussed.

Dr. K. Seamon of the FDA reviewed the US regulatory guidelines. Recombinant proteins do not have to be identical with the natural congeners, since this not feasible in view of natural variability. However, proof of manufacturing reproducibility is very important. He illustrated his talk with examples encountered during erythropoiten and TPA registration.

Dr. Sandow presented an overview of the pharmacokinetics of several peptides and proteins in clinical use, and the many factors which govern their disposition. For many of these products, a wealth of information has become available in the last ten years.

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Dr. J. Verhoef gave an overview of problems encountered when trying to make peptides and proteins cross biological membranes. Although many penetration enhancers have been tried, only very few lack irritant properties. Dr. Verhoef showed results of studies done with the relatively non-irritating enhancer, dimethyl-beta cyclodextrin. This compound promotes absorption of insulin and other peptides through the nasal epithelium, while only moderately inhibiting ciliary movement.

Dr. R. van Breemen presented an elegant screening method for evaluating the hydrolysis of peptides using immobilized proteases. The method correlates well with results obtained in vivo. This technique can be used to identify the most stable peptides and would result in a decreased use of experimental animals.

Dr. A. Warrander showed how the combination of labeling and analytical techniques, notably tandem MS-MS, were utilized to elucidate the metabolic fate of a decapeptide LHRH analogue. Trace quantities of metabolites were identified in biological matrices of several species including man.

Session 12: Biotransformation and Biomonitoring

Dr. P. Farmer discussed various techniques, mainly GC-MS, for monitoring human exposure to genotoxic compounds. Dr. Farmer uses hemoglobin (Hb) adduct formation to assess the formation of chemically reactive molecules. The different approaches were illustrated with examples of occupational exposure to styrene oxide, ethylene oxide and 4,4'-methylene dianiline.

Dr. N. Vermeulen gave a description of biomonitoring by GC-MS analysis of mercapturic acids excreted in the urine. Apart from dose assessment, the urinary kinetics may reflect the toxicokinetics of the electrophilic metabolites. Examples included occupational exposure to the nematocide 1,3-dichloropropene, and a study of benzene metabolic pathways.

Dr. N. van Sittert noted that biomonitoring may serve multiple purposes. It can be used to assess exposure to chemicals, and determine the biological fate of chemicals. Practical examples included biomonitoring of workers occupationally exposed to benzene and ethylene oxide by measuring urinary excretion of S-phenyl-mercapturic acid and formation of hydroxyethyl valine in hemoglobin, respectively.

Alternative biomonitoring methods were described by Dr. H. Bolt. Ethylene oxide and vinyl chloride exposure were measured by GC-MS analysis of TMS derivatives of the respective 7-(2-hydroxyethyl)-and 7-(2-oxoethyl) guanine adducts. The vinyl chloride adduct was shown to be much more genotoxic.

Formation of genotoxic electophilic metabolites from O-alkyl substituted neurotoxic tri-arylphosphates was discussed by Dr. A. Mentzschel. Various o-hydroxybenzyl substituted adducts were formed from a reactive benzodiozaphosphoron metabolite of tri-(o-methylbenzyl)phosphate when reacted with guanosine, cytidine or DNA in vitro.

Session 13: Disposition of Metals and Metal-contining Drugs

In the first lecture of this session, Dr. C. Klaassen posed the question why animals die from liver injury after acute Cd exposure while they die from kidney injury following chronic Cd exposure. It was explained that after acute administration, Cd is predominantly found in liver cytosol where it is bound to metallothionein (MT). The Cd-MT is released from the liver into the plasma and is then taken up by the kidney. Subsequent dissociation of the Cd-MT may explain the chronic nephrotoxicity since the kidney synthesizes less MT than the liver. Interestingly, MT is induced by Cd as well as alkylating agents, cytokines, alcohols and agents that induce oxidative stress.

Dr. R. Kelland discussed a novel class of antitumor drugs, the platinum IV amine/amine dicarboxylates. Members of this class were active in various tumor cells which were resistant to cisplatin. Also, cells which were made resistant by exposure to low doses of cisplatin were still sensitive to these new compounds. These drugs provide an interesting lead to circumvent cisplatin resistance. A phase I clinical trial has begun with one of these compounds. Biotransformation studies indicate that 30% of the Pt-compound is not recovered.

Dr. A. Penninks discussed the absorption, tissue distribution and excretion of the organotin compounds, 14C-di-n-octyl-tin-dichloride and 14C-tri-n-butyl-tin-acetate, in relation to the immuno-toxicity they produce in rats.

Studies presented by Dr. J. Groten described the different disposition of Cd in rats when administered in the diet as $CdCl_2$ or Cd-MT. The ratio of Cd in kidney to Cd in liver is higher after administration of Cd-MT. Ferrous ions in the diet protected against Cd accumulation.

The results presented by Dr. E. Gieichmann suggest that the adverse side-effects of gold (I) compounds are caused by gold (III), which is gradually formed in vivo. Gold (III) is chemically highly reactive, binds to protein thiols, forms cross-links and precipitates. Myeloperoxidase in phagocytic cells might be involved in the oxidation of gold (I). T-cell responses to gold (III) after administration of gold (I) were used as an elegant detection mechanism for the formation of traces of gold (III).

The Third International Meeting in Amsterdam was a tremendous success, and upheld the traditions and standards of ISSX. The science was excellent and timely. As always there were wonderful opportunities for meeting old friends and making new ones. And there is more to come! Next year the 4th European ISSX meeting will be held in Bologna, Italy and the 4th North American meeting will be held in Bal Harbour, Florida. Cherish good memories and great expectations. See you then!

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ISSX Council Meeting

(Contributed by Nancy Holahan, ISSX Administrative Officer)

The 1991 ISSX Council Meeting took place June 26 at the Bruin Cafe on the Free University campus in Amsterdam during the Third International ISSX Meeting. A total of thirty-two members attended, including nineteen voting Officers and Councillors, our Administative Officer, Newsletter Editor, Committee Chairpersons, Past Presidents and ISSX meeting organizers.

In his opening remarks President Franz Oesch thanked the organizers of the ongoing international meeting and welcomed the organizers of future scientific meetings who were present as guests. He emphasized that high quality meetings are the Society's most important function. Oesch also commented on the strong response to the course offered on regulatory affairs and noted that our revised Constitution and By-Laws represent a major accomplishment of the past year.

The Constitution and By-Laws Committee, chaired by David Yesair, reported that the revised document was approved by more than 99% of the voting members and participation in the election was high with a 46% response rate.

The Treasurer's Report, presented by Fred Pritchard, ISSX Treasurer, indicated that the Society's financial condition is healthy and dues collected from new members are ahead of projections. Pritchard noted that there will be no dues increase for 1992. Successful corporate fundraising for our meetings has made it possible to keep membership dues low, since dues alone do not cover our operating expenses. The Society's financial status during the first ten years of its existence reflects healthy growth.

Luciano Vittozzi and Larry Kaminsky, the organizers of the 1992 European ISSX Meeting (Bologna, Italy, July 4-6) and the 1992 North American ISSX Meeting (Bal Harbour, Florida, November 2-6), respectively, reported on the status of plans for those meetings. They are giving top priority to making the meetings affordable for students by offering reduced registration fees. Luciano Vittozzi announced plans to provide free registration for 20 students.

Council also approved Tuscon, Arizona as the site for the 1993 North American Meeting and Seattle, Washington for the 1995 Fourth International Meeting. Tentative plans are underway for a European regional meeting in France during 1993. Sweden was named as a possible location for European meeting in a future year. John Caldwell, Chairman of the Scientific Affairs Committee (SAC), indicated that the SAC is striving to schedule meetings five to six years in advance.

David Hutson reported that ISSX is co-sponsoring with the Agrochemicals Division of the American Chemical Society (ACS) a two-day symposium on the fate of xenobiotics in food-producing animals. This symposium will be held August 28-29, 1991, in New York City as part of the 202nd National meeting of the ACS.

Membership Affairs Committee Chairman Ernie Hodgson reported that membership has more than doubled since 1984 but that additional effort is needed to attract new members from countries outside the USA. All members are encouraged to use the membership application form provided with each issue of the Newsletter in order to acquaint their colleagues with ISSX. The committee has begun a drive to recruit more student members.

Hodgson reported that contributions made by members to the Membership Fund were sufficient to support 30 new members in 1991. He hopes the Fund will be able to continue to provide support in future years.

The Publications Committee, chaired by Guy Paulson, is investigating options for the publication of citable abstracts from ISSX meetings as an added benefit to all members.

Newsletter Editor Martin Dyroff announced that the June issue is being delayed in order to include coverage of the June 24-28 International ISSX meeting in Amsterdam. The election ballot was mailed in June as planned. Dyroff encouraged all Committee Chairpersons and more members to become involved with the Newsletter by contributing items of interest, large or small.

David Case, Chairman of the Committee on Regulatory Affairs (CORA), noted that his Committee organized a session at the last two ISSX meetings and asked how frequently such sessions should be held. It was agreed that every ISSX meeting should have a session on regulatory affairs and that regulators should be included in order to broaden the debates.

Nominating Committee Chairman Neal Castagnoli, Jr. reported that he was very pleased with the response from those asked to run for office. He noted that this is a positive sign for the Society. It was suggested that when appointments are made to fill committee positions, unelected candidates should be considered, as well as members suggested by the general membership.

Gerard Mulder, Chairman of the Organizing Committee of the Third International ISSX Meeting in Amsterdam, reported that 540 had registered for the meeting.

Before adjourning, the Council meeting participants thanked our host Nico Vermeulen for arranging such a pleasant location for the 1991 Council Meeting.

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ISSX Business Meeting

(Contributed by Nancy Holahan, ISSX Administrative Officer)

President Franz Oesch welcomed all present at the annual ISSX Business Meeting which was held June 27, 1991 at Free University, Amsterdam, and told them he was happy to share news from the Council Meeting held the previous evening. A review of the Society's financial situation since its inception in 1981 showed steady growth and our Treasurer declared us to be on solid financial ground. Reporting that 873 of our 1430 members are from the USA, Oesch called on members to spread the word about ISSX, particularly to their colleagues outside the USA.

Next, President Oesch shared a Publications Committee proposal to publish citable abstracts for ISSX scientific meetings and distribute them to all ISSX members, regardless of meeting attendance. These abstracts would either be published by the Society or as a supplement to an established journal. Oesch commented that extended abstracts were being considered. He then opened the floor for discussion.

It was noted that publication of meeting abstracts is a sign of the Society's maturity. Questions raised included whether publication of the abstracts would be paid from meeting budgets or from general Society funds, whether the abstracts should be reviewed for content and value or merely for format, whether extended length would prohibit authors from presenting new work or subsequent publication of full papers, and whether abstract books should be distributed to meeting participants prior to the meeting. President Oesch concluded that all were in agreement that we should have citable abstracts and that this would be reported to the Publications Committee, who would then be requested to present a detailed proposal to Council.

President Oesch pointed out that the course offered by the Committee on Regulatory Affairs (CORA) at the present meeting was overbooked, indicating strong demand. With continual revisions in regulations and an increasing number of areas subject to regulation, it is useful for members to have frequent forums for discussion of this topic. Oesch asked how often sessions on regulatory issues were desired. The consensus was that yearly sessions would be very useful.

Regarding recruitment of members for Committee positions, President Oesch announced that the next issue of the Newsletter would include a form for suggesting candidates for Committee positions -- either oneself or another. Members can also send suggestions to our President, President-Elect or Administrative Officer.

Addressing the issue of reduced registration fees for students at ISSX meetings, President Oesch stated that Council agreed that fees should be reduced as much as possible for students who are ISSX members. For the July 1992 European meeting to be held in Bologna, Italy, student fees have been reduced to $150, which will cover only direct costs such as lunches and printing of materials. The student registration fees will not cover other costs, such as speakers' expenses. It was suggested that corporate funds might be used to pay for student travel and that corporations might contribute to a five year student bursary. One Councillor suggested that the Society adopt reduced meeting registration fees for retired persons. The Membership Affairs Committee will be asked to explore this suggestion as an added benefit of Emeritus membership.

President Oesch reported that 46% of the membership had returned ballots on the adoption of the revised Constitution and By-Laws and that the revisions had been overwhelmingly approved by more than 99% of those voting. One member suggested that the By-Laws be amended to state that any subdivisions of the society (by discipline or region) be overseen by Council. Oesch reported that this amendment will be presented for vote in an upcoming issue of the Newsletter.

President Oesch then turned over the microphone to President-Elect Mitchell Cayen, who thanked our current President for his fine leadership and went on to announce that he would begin forming new Committees this September and October. Cayen plans to widen the range of participants and underscored the earlier suggestion that members are encouraged to volunteer for a Committee or to suggest a colleague. Cayen specified that the membership of the Publications, Membership, Finance, Scientific Affairs and Regulatory Affairs Committees would be changing and that he was looking for volunteers in the next 2-3 months to help form a pool of candidates.

President Oesch pointed out that the Newsletter is one of the most difficult jobs in the Society. He told members that the ballot for the election of officers had already been mailed and that the June issue has been postponed to include coverage of the meeting in Amsterdam. Newsletter Editor Martin Dyroff called for meeting reports, book reviews, commentaries, complaints, letters to the editor and anything else associated with xenobiotic research. He urged members to send material for the Newsletter and added that he was looking forward to working with all members, as well as all elected officials -- and those to be elected.

Past President Ron Estabrook made the point that he hoped to see more women on the programs of our future meetings. ISSX represents both sexes, he emphasized.

President Oesch ended the Business Meeting by thanking Gerard Mulder and Nico Vermeulen and the entire Organizing Committee of the Third International ISSX Meeting for their fine efforts on behalf of the Society.

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Meeting Reports

XMTWBC '91

(Contributed by Dr. M. Mikov)

The Xenobiotic Metabolism and Toxicity Workshop of the Balkan Countries (XMTWBC) was held from March 18 - 21, 1991 at the Medical Faculty Novi Sad and the Hotel Park, Novi Sad, Yugoslavia. This Workshop was organized and supported by the Drug Metabolism Section of the International Union of Pharmacology. ISSX supported the attendance of young scientists from Balkan countries.

The workshop was opened by R.L. Smith who gave an introductory lecture on the significance of genetic polymorphism of drug metabolizing enzymes for adverse drug reactions and human idiopathic disease.

A. Benakis reviewed the use of labelled compounds in xenobiotic metabolism studies. He also gave practical demonstrations of the aminopyrine breath test using newly designed metabolism cages and the use of caffeine for the determination of acetylator phenotype. M. Marselos proposed using two inducible hepatic aldehyde dehydrogenases as bio-indicators of exposure to xenobiotics.

One afternoon session was devoted to the EEC COST Project B1, Round II: "The role of metabolic and kinetic studies in drug research and development". A. Klose described the COST mechanism as a framework for successful European cooperation in the field of research and development. Several participants in the COST program gave accounts of their activities. A. Boobis discussed past and future uses of in vitro drug metabolism studies and O. Pelkonen reviewed ways to determine the significance of non-genetic factors for human interindividual variation in drug metabolism. M. Stanulovic chaired a round table discussion on drug metabolism regulatory requirements.

J.P. Thenot gave a lecture on applications of biomedical mass spectrometry and E.H. Jansen spoke on modern analytical methods in biochemical toxicology. R.L. Smith gave an overview on stereochemical aspects of drug metabolism, L. Vereczkey described the role of biotransformation in toxicology and S. Yanev reviewed inhibitors of cytochrome P-450 from toxicological considerations to therapeutic benefits.

Oral and poster presentations were made by scientists from the region. M. Iscan described the differential response of rodent hepatic xenobiotic metabolizing enzymes to acute nickel treatment. S. Krstev discussed the consequences of long term exposure to carbon disulfide and M. Mikov described the in vitro metabolism of paracetamol-3-cysteine by caecal contents. I. Cok et al. presented a poster on acetylation phenotype in patients with breast cancer, S. Dobric and A. Lathinovic discussed the influence of the radioprotector WR-2721 on acute paracetamol toxicity in the rat. P. Pappas et al. showed that hepatic glutathione depletion affects the induction of aldehyde dehydrogenase while M. Karageorgou showed sex differences in the induction of aldehyde dehydrogenase by methylcholanthrene. J. Popovic proposed predicting acenocoumarol systemic availability from plasma level data after oral administration to man and V. Jakovljevic presented a method for the detection of metoprolol in body fluids.

It is anticipated that the next Xenobiotic Metabolism and Toxicity Workshop of the Balkan Countries will be held during 1993 in Ioannina, Greece. Professor M. Marselos will be the local organizer.

The proceedings of this Workshop will be published as a supplement to *The European Journal of Drug Metabolism and Pharmacokinetics*. A copy of the book of abstracts may be obtained from:

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Australian and New Zealand Environmental Mutagen Society

The annual meeting of the Australian and New Zealand EMS was held in Adelaide, Australia, November 22 - 23, 1990. The meeting was opened by the State Minister of Health and featured a wide variety of topics ranging from the effects of pesticide and herbicide usage in Australia to detailed studies on the mechanisms of mutagenesis.

The program was organized into three areas: biochemical, genetic and environmental toxicology. The biochemical toxicology section dealt with the identification of cytochrome P450 and UDP glucuronosyltransferase isozymes involved in carcinogen metabolism and the role of these enzymes, glutathione transferase and N-acetyltransferase in the etiology of human cancer (McManus, Mackenzie and Sanderson, Adelaide; Minchin, Perth). The genetic toxicology section focussed on the mechanisms of mutagenicity and tumor promotion (Murray, Dempsey, and Record, Adelaide; Ferguson, Auckland) including the role of mobile DNA elements (Saint, Adelaide) and methods for detecting genotoxic events (Baker, Sydney; Ford and Fenech, Adelaide). The means of measuring exposure to mutagens was also a focus of the environmental toxicology section where changes in the sexual characteristics of welks (Chapman, Sydney) and deformities in frog limbs and midge larvae (Tyler and Madden, Adelaide) were demonstrated to reflect the level of mutagenic pollutants in the aquatic environment. The pervasive and detrimental effects of the use of herbicides and the insecticide, dieldrin, were also discussed (Preston, Calder and Gun, Adelaide).

The Sixth International Conference on Environmental Mutagens will be held in Melbourne, Australia, February 21-26, 1993.

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Book Review

(Dr. L.A. Damani has edited a comprehensive series on the biochemistry, metabolism, pharmacokinetics, and toxicological/ pharmacological aspects of sulfur-containing compounds, both natural and synthetic. This treatise consists of 3 volumes, each in two parts. The first volume is reviewed in this issue, and the remaining two volumes will be reviewed in the next issue of the Newsletter.
Book Review Editor: Fred Falkner)

Sulphur-Containing Drugs and Related Organic Compounds: Chemistry, Biochemistry, and Toxicology. Volume 1: Metabolism of Sulphur Functional Groups, Part A (167 pp) and Part B (324 pp), Ellis Horwood, Chichester, West Sussex, England (distributed by John Wiley worldwide), 1989. Each Part $63.

Reviewed by Dr. Kishin J. Kripalani, Associate Director of Metabolism and Pharmacokinetics, Bristol-Myers Squibb (BMS) Research Institute, Lawrenceville, NJ, USA. Dr. Kripalani is a graduate of the University of Bombay and received his Ph.D. from the University of California, San Francisco. As an Associate Director at BMS Research Institute, Dr. Kripalani is responsible for metabolism studies during the development of new drugs and studies of pharmacokinetic and pharmacodynamic mechanisms. He has also conducted pioneering work in the area of metabolic inversion of arylpropionic acids. Dr. Kripalani is a member of ISSX, ACS, ASPET, and AAPS.

This is the first of a three-volume series of books intended to review current knowledge concerning chemical mechanisms of biotransformation and drug toxicity. The material on sulfur xenobiochemistry is comprehensively organized in these three volumes to serve as a reference source on the subject. The editor has authored or co-authored a few of the chapters; the remainder of the chapters are contributions from numerous experts in the field. The first volume (which is the subject of this review) is subdivided into Parts A and B. Part A provides an overview of the biotransformation of natural and synthetic sulfur-containing compounds which include agricultural, industrial, and medicinal agents, and briefly summarizes Phase I and Phase II metabolism of various sulfur functional groups.

Together, parts A and B cover the chemical and biochemical reactivity of organic and, to some extent, inorganic compounds having different types of sulfur-containing functional groups.

Chapter 1, as an editorial introduction, highlights main features of sulfur chemistry and biochemistry (i.e., metabolism of endogenous sulfur-containing compounds), and provides a brief historical overview of the metabolism of exogenous sulfur-containing compounds and the possible metabolic reactions that various sulfur compounds may undergo depending on the functionality of the sulfur group. Tabulated data on "metabolic options open to sulfur functionalities" in this chapter provide a useful way to attempt to make reasonable predictions of the likely metabolic profiles or rationalize observed biotransformations.

Chapter 2 is concerned primarily with the biologically essential sulfur-containing compounds, and sulfur-containing antibiotics. Although not a very comprehensive account, this chapter, nevertheless, provides key references for each group of compounds. This chapter highlights the significance of chemical properties of organic sulfur compounds as determinants of their biochemical function.

Chapter 3 deals with a variety of compounds in agricultural use (pesticides, herbicides, etc.) that contain sulfur. A fairly large number of examples are presented to delineate a variety of mechanisms of action of these compounds. These examples include oxidative bioactivation which can generate reactive molecules by elimination of sulfur or activation of the constituent molecules by generating readily displaceable sulfur-containing leaving groups, and hydrolysis to generate biologically active thiophenols. Metabolism of these compounds in plants and microorganisms is briefly discussed.

Chapter 4 appears to be an introduction to subsequent chapters and points out the widespread use of sulfur as organic and inorganic derivatives in industry and medicine and the consequent exposure of most biological systems to sulfur and sulfur-containing compounds; therefore there exists a need to understand the effect and fate of sulfur-containing compounds in vivo. The section on agricultural agents is repetitive.

Chapter 5 presents a reasonably comprehensive account of mammalian metabolism of inorganic sulfur compounds which includes: inorganic sulfate, sulfite, sulfur dioxide, polythionates, thiosulfate, thiocyanate, elemental sulfur and sulfide.

Chapter 6 is a rather brief account of chemical, analytical, and biochemical aspects (Phase 1 metabolism) of thioethers, thiols, dithioic acids and disulfides. Pharmacological and toxicological aspects are briefly discussed.

Chapter 7 contains a short review of metabolic conjugation of thiol and dithioic acid groups. Individual classes of compounds are not discussed in Volume 1, but are covered in subsequent volumes.

Part B of Volume 1 provides a thorough and comprehensive account of several classes of sulfur-containing compounds and follows up on the chemical and mechanistic theme of Part A. Topics include metabolic reactions, enzymes involved in these reactions, and molecular mechanism of toxicity.

Chapters 1 through 3 discuss the processes involved in desulfuration of phosphothiorates, thioamides, carbamothioates and dithioates. Comparison of metabolic reactions of carbamothioates by microoganisms, plants, animals, and in the

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environment (i.e., photodegradation) is presented in a tabular form.

Oxidation and reduction is presented in chapter 5; the possibility of reduction of sulfones to sulfoxides is also examined. The role of both tissue and microbial enzyme systems responsible for these reactions is covered. This chapter also compares the pharmacological and toxicological activities of the different redox states of the sulfoxide group.

Chapters 6 through 8 proceed to describe similar aspects of further metabolism of sulfonium salts, sulfonamides, and sulfamates, sulfonates and sulfate esters. All the chapters are thorough and well referenced. Volume B concludes with chapters reviewing the metabolism of sulfur heterocycles (ch. 9) and sulfur-containing secondary metabolites (ch 10).

Overall, the editor has succeeded in the difficult task of compiling a cohesive and thematic work that will serve as a useful reference for current knowledge in sulfur xenobiochemistry.

New Journals

The following new journals have recently been announced or issued:

Inhalation Toxicology
Editor: D.E. Gardner
Publisher: Taylor & Francis; New York, Washington, Philadelphia, London

Current Opinion in Therapeutic Patents
Publisher: Current Patents, Ltd.; London

Journal of Pharmaceutical Medicine
Editors: B. Dickson, M. Young
Publisher: Blackwell Scientific Publications; Oxford, UK

Pharmacology Communications
Editors: N. Bowery, R. Ruffolo
Publisher: Harwood Academic Publishers; Chur, London, Paris, New York

ISSX Committee Membership

All ISSX members are encouraged to nominate candidates for positions on ISSX Committees. It was recommended at the 1990 ISSX Council Meeting, and again supported at the 1991 Council Meeting, that recommendations be solicited from the membership at large in order to broaden the involvement of our membership in the operation and activities of our Society. Our incoming President, Mitchell N. Cayen, will be making appointments to the following ISSX standing Committees this fall:

Committee on Regulatory Affairs (CORA)
Executive Committee
Finance Committee
Membership Affairs Committee
Publications Committee
Regional Contributing Editors for the Newsletter
Scientific Affairs Committee

It is acceptable to nominate yourself or another member of ISSX. If you suggest someone else, please confirm that the individual is willing to serve as a member of the committee.

Nominations may be sent to either our Administrative Officer, Nancy Holahan, or the President-Elect, Mitch Cayen, at the following addresses:

Ms. Nancy Holahan
ISSX Administrative Officer
PO Box 3
Cabin John, MD 20818, USA
Fax: (302) 983-5357

Dr. Mitchell N. Cayen
ISSX President-Elect
Schering Plough Research
60 Orange Street
Bloomfield, NJ 07003, USA
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ISSX Constitution and By-Laws

A total of 641 members returned their ballots concerning the revised ISSX Constitution and By-Laws. This represents a response by 46% of our membership. The revisions were approved overwhelmingly by 99.7% of the respondents. A few members also sent helpful comments and suggestions. At the 1991 Council Meeting it was recommended that one of these suggestions be put to a vote by the general membership. Thanks to those who participated in this important Society activity.

Membership Drive

We continue to distribute ISSX membership brochures at meetings as a primary means of soliciting new members. If you would be able to assist the Society by placing brochures at the registration tables of meetings you will be attending, kindly contact Ms. Nancy Holahan, ISSX Administrative Officer, and we will be pleased to forward a supply of brochures. We are particularly anxious to increase the international representation of our membership.

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People and Places

Awards for Dutch Scientists

(Contributed by Dr. Nico P.E. Vermeulen)

Dr. Gerard Mulder

The Hildegard Doerenkamp and Gerhard Zbinden Foundation for Realistic Animal Protection in Scinetific Research (Zurich, Switzerland) has awarded a special prize to Dr. Gerard J. Mulder, Head of the Division of Toxicology of the Center for Bio-Pharmaceutical Sciences (Leyden University) for the work he and his group (in particular Dr. J. Fred Nagelkerke) have done in the area of in vitro toxicology. This Foundation gives an annual award of 50,000 German Marks ($120,000) for important contributions in the area of alternatives to animal experiments. Professor Mulder's group has developed a test system for detection of reactive intermediates in drug metabolism. In addition, the use and culture of isolated kidney tubular cells and hepatocytes for mechanistic toxicology studies is a major theme in his lab. During 1990 two Ph.D. theses on these subjects were completed:

P. Dogterom, Ph.D.: *Cytotoxicity and protein thiol modifications in isolated rat hepatocytes.*

P.J. Boogaard, Ph.D.: *Development of proximal tubular cell systems to study nephrotoxicity in vitro.*

The Division of Toxicology also organizes an international course on in vitro test systems. The most recent course was held in April 1991.

Dr. Glenn S. Baldew

Last September Dr. Glenn S. Baldew received the M.F.R. Pharmacochemistry Award from the Dutch Section of Pharmacochemistry of the Royal Dutch Chemical Society. This award (Dfl. 5,000) is provided by the Foundation of Medical and Pharmaceutical Research (M.F.R.) and is awarded every two years to the writer of the best Dutch Ph.D. thesis in the area of Medicinal Chemistry. The title of Baldew's thesis was:

"Chemoprotection against cis-Platin-induced nephrotoxicity: A molecular approach with selenium compounds".

His Ph.D. supervisors were Prof. Dr. N.P.E. Vermeulen (Vrije Universiteit, Amsterdam) and Prof. Dr. IrJ.J.M. de Geoij (Inter-University Reactor Institute, Delft).

Dr. Guido Haenen

In February 1991 Dr. Guido R.M.M. Haenen received a Royal Dutch Shell Study Award from Shell Research BV. This award (Dfl. 10,000) is provided yearly to a young talented scientist working at a Dutch University and is intended to enable the scientist to visit laboratories and scientists abroad. Haenen's research interest has focused on the role of thiols in oxidative stress with emphasis on catecholamine toxicity. He is presently working as a scientific researcher in the Department of Pharmacochemistry, Vrije Universiteit, Amsterdam.

Dr. Fred Deckert; VP-Elect of Mid-Atlantic SOT

Dr. Fred Deckert was recently elected Vice-President-Elect of the Society of Toxicology/Mid-Atlantic Chapter (MASOT). He will be the Vice President of MASOT in 1992, and the President in 1993-1994.

MASOT was founded 10 years ago to serve as a focal point for toxicologists in the region; encourage interactions among toxicologists in government, industry and academia; and sponsor scientific and educational programs in toxicology. An MASOT symposium on Developmental Toxicology will be held October 10, 1991.

Towards our International Society

As the Regional Contributing Editor from Belgium, France and Switzerland I would like to take this opportunity to make some comments on the *State of the Society* article written by Nancy Holahan and published in the March 1991 issue of the Newsletter.

I was pleasantly surprised by the growing number of ISSX members in Belgium, France and Switzerland: +28% in 1990 with nearly 100 members. Germany and Japan have also increased their membership by 25% (40 members) and 16% (78 members), respectively.

This is very good news indeed for President Franz Oesch who expressed his desire for membership to increase in a truly international fashion (Newsletter, December 1989). Although membership from the USA still accounts for 62% of all ISSX members, scientists from other countries, especially in Europe, have begun to be more involved with ISSX.

As a Regional Editor for the Newsletter, I urge the 92 members from Belgium, France and Switzerland to serve the very important function of keeping other members informed of events and research activities in our part of the world. I would also like to share with you my enthusiasm for the Newsletter, so take your pens and send me your contributions: every event in connection with the study of xenobiotics is of interest.

To extend what Past-President Ron Estabrook said in his message from the President's Desk (ISSX Newsletter, September 1989):

"If every ISSX member from Belgium, France and Switzerland were to recruit a new member in the next year, our ranks would swell to over 200 members. Let's do it!!"

Andre Weil

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